Reports of Practical Oncology and Radiotherapy 25 (2020) 323–326

Available online at www.sciencedirect.com

Reports of Practical Oncology and Radiotherapy

journal homepage: http://www.elsevier.com/locate/rpor

Review

Cancer cells resist hyperthermia due to its obstructed activation of caspase 3

Xiaoren Tang[a,*], Feng Cao[a], Weiyuan Ma[b], Yinian Tang[c], Bushra Aljahdali[a], Mansour Alasir[a], i Erdjan Salih[a], Serge Dibart[a]

[a] Henry M. Goldman School of Dental Medicine, Department of Periodontology, 650 Albany Street, Boston, MA, USA
[b] Department of Neurology, Massachusetts General Hospital, Harvard Medical School, 185 Cambridge Street, Boston, MA, USA
[c] School of Medicine, Boston University, 72 East Concord St, Boston, MA, USA

ARTICLE INFO

Article history:
Received 13 February 2019
Received in revised form 16 January 2020
Accepted 19 February 2020
Available online 26 February 2020

Keywords:
Cancer cells resist hyperthermia

ABSTRACT

Aim: It is well known that inducing hyperthermia is a type of cancer treatment but some research groups indicate that this treatment is not effective. This article finds and explains the mechanism of this treatment and its possible problems.
Background: Hyperthermia is commonly known as a state when the temperature of the body rises to a level that can threaten one's health. Hyperthermia is a type of cancer treatment in which body tissue is exposed to high temperatures (up to 45 °C). Research has shown that high temperatures can damage and kill cancer cells, usually with minimal injury to normal tissues. However, this mechanism is not known.
Materials and Methods: We recently treated cancer cells with different temperatures ranging from 37 °C to 47 °C and further measured their caspase 3 secretion by ELISA, western blot and cell survival rate by microscope.
Results: We found that most cancer cells are able to resist hyperthermia more than normal cells most likely via non-activation of caspase3. We also found that hyperthermia-treated (≥41°) cancer cells extend a long pseudopod-like extension in comparison to the same cancer cells under normal conditions.
Conclusion: Our data here indicates that cancer cells have resistance to higher temperatures compared to normal cells via non-activation of caspase 3. This is a significant issue that needs to be brought to attention as the medical community has always believed that a high temperature treatment can selectively kill cancer/tumor cells. Additionally, we believe that the pseudopod-like extensions of hyperthermia-treated cancer cells must be related to its resistance to hyperthermia.

1. Introduction

Out of many therapies for cancer treatment, treating hyperthermia (also called thermal therapy or thermotherapy) has become very important.[1–3] Some studies that have investigated the mechanism of hyperthermia have found that this treatment significantly damages and kills cancer cells[4–6] via inhibition of cell growth[7] or activation of cell apoptosis due to induction of caspase 3.[8,9] However, there is no further experimental data to clarify how cancer cells are more sensitive to hyperthermia than normal cells, nor is there adequate information and understanding of the mechanism itself. In this paper, we found that in contrast to prior research, cancer cells are able to resist hyperthermia more than normal epithelial cells and mouse macrophages. We also found that this phenomenon is due to cancer cells' non-activation of caspase 3, even though it is known that hyperthermia (43 °C)-treated cells induces caspase-3 activation.[10]

2. Material and method

2.1. Cells

RAW 264.7 cells (TIB-71, ATCC) in a RPMI-1640 media (REF#: 11875–093, Life Technologies, NY) with 10% FBS, mtsv1-7 Cat#: 10081202, Sigma, U2OS HTB-96, ATCC, A549 CCL-185, ATCC, CAL27 CRL-2095, ATCC, or PC-3 CRL-1435, ATCC cells in a DMEM media REF#:11965–084, Life Technologies, NY with 10% FBS were cultured at 37 °C in a 5% CO2 atmosphere.

* Corresponding author.
E-mail address: xrtang@bu.edu (X. Tang).

https://doi.org/10.1016/j.rpor.2020.02.008
1507-1367/

Table 1
Cell survival rate after temperature treatment. The pre-cultured cells (each type) with ≥90% cell confluent in the well of 6 well/plate were washed with pBS and treated with 250 μl of 0.25% trypsin for 5 min, then mixed with 2 ml of cell culture medium. Cells were collected, transferred into the sterilized 15 ml tube, and incubated in a water bath at 37 °C (control) or temperatures of 40 °C, 41 °C, 42 °C, 43 °C, 44 °C, 45 °C, 46 °C or 47 °C (test group) for 1 h. The treated cells with medium were re-plated into one well of a six well/plate and cultured at 37 °C, 5%CO_2 for 24 h. A microscope was used to monitor cells. After culturing cells for 24 h, cell survival rate was calculated and graphed.

Table 1. Cell survival rate after temperature treatment.

Name	Type	Tissue	Disease	Survival rate (%)								
mtsv1-7	Human breast	epithelia	N/A	100	64.5	48.2	23.7	1.6	0	0	0	0
RAW	macrophage	ascites	N/A	100	34.5	2.3	0	0	0	0	0	0
U2OS	epithelial	Bone	osteosarcoma	100	100	100	90.2	50.4	15.5	5.2	0	0
A549	epithelial	Lung	carcinoma	100	100	100	92.6	85.4	73.1	54.1	3.3	0
CAL27	epithelial	tongue	carcinoma	100	100	80.3	50.5	15.2	1.7	0	0	0
PC-3	epithelial	prostate	adenocarcinoma	100	100	90.2	50.4	25.3	10.3	6.6	0	0
Cells				37°C	40°C	41°C	42°C	43°C	44°C	45°C	46°C	47°C
				Cell treated at different temperature								

2.2. Cell treatment and analysis

Pre-cultured cells with ≥90 cell confluence/plate (6 well plate, Sigma) were collected by trypsinization, washed with pBS and added to 1 ml fresh media in a 50 ml conical screw cap tub (usa-scientific.com), and then treated in a water bath with different temperatures ranging from 37 °C to 47 °C for 1 h*. Cells were then respectively used for:

1 ELISA. The media from each treated cells were subjected to caspase 3 detection with a kit (Cat#:AB181418, Abcam). ELISA immunoreactivity was quantified using a microplate reader (Model 680, Bio-Rad). The data were analyzed and then graphed.
2 Western blot. The protein purified from each treated cells were detected by Western blot with caspase 3 antibody (sc56053, Santa Cruz Biotechnology) following the manufacturer's instructions.
3 Calculation of cells survival rate. The treated cells were added to 1 ml RPMI-1640 fresh media in one well of 6 well plate and re-cultured at 37 °C in a 5% CO_2 atmosphere overnight. Cells were observed under 1 × 10, 1 × 20 microscope. The surviving cells with the highest confluence per one view of (1 × 20) observation from each group were selected and their cell numbers were counted. Survival rate = the number of cells that survived from each test group treated in a hyperthermic state at >37 °C/the number of survived cells (same cell type) treated in normal conditions at 37 °C.

* It is known that cell growth requires incubation at a certain temperature and with 5% CO_2, but when we tried to treat cells with various temperatures, we found that the incubator could not display an instant and accurate temperature measurement. Thus, the water bath was a solution to this issue. However, with water bath treatment, the cells cannot survive lacking 5% CO_2 for more than an hour, (according to the experience of our laboratory, cells can still grow normally within one hour of lacking 5% CO_2 [data not shown]) and so treatment was limited to one hour.

2.3. Statistical analysis

All experiments were performed in triplicate and statistical analyses were conducted with the SAS software package. All data were normally distributed. For multiple mean comparisons, we conducted analysis of variance (ANOVA) and the Student's *t*-test for single mean comparison. For time-course study, we used a two-way repeated measure ANOVA. P values lower than 0.05 were considered significant.

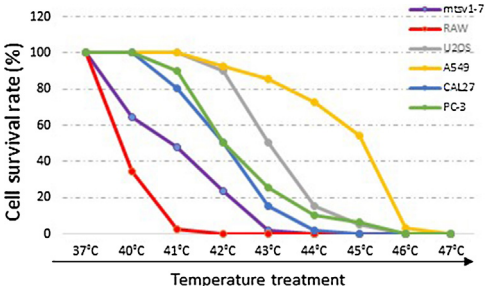

Fig. 1. Hyperthermia assay. The survival rate of cancer cells was compared with the control cells and mapped.

3. Results

In order to examine the effects of hyperthermia on cancer cells versus normal cells, mtsv1-7 cells (normal human breast epithelial cells), RAW264.7 cells (normal mouse macrophage-like cells) as the control group, and cancer cells (U2OS, A549, CAL27, or PC-3) as the test group were treated with different temperatures ranging from 37 °C to 47 °C. The treated cells were analyzed. The medium from each group were collected and subjected to ELISA (anti-caspase 3), the number of cells growing on the plate were counted, and their survival rate was calculated. As shown in Table 1, ≥98% of mtsv1-7 (the normal human epithelial cells) had died after 43 °C treatment for 1 h and completely died when the temperature increased to 44 °C for 1 h. Mouse macrophage-like cells (RAW264.7) were at 100% death only after 1 h 42 °C treatment. However, because four cancer cells survived at 44 °C, and A549 (lung cancer cells) was at 3.3% survival after 46 °C treatment, its survival rates were compared and mapped (Fig. 1). Since hyperthermia treatment may not completely kill the free cancer cell in media or blood vessels, we hypothesized that hyperthermia induces cell death via activation of caspase 3 only in normal cells rather than in cancer cells. To test this hypothesis, analysis of caspase 3 after hyperthermia treatment in cells was carried out. As shown in Fig. 2, the control group cells significantly induced caspase 3 production after 41 °C treatment, but at the same condition, all four cancer cells did not strongly release caspase 3. Among the four cancer cells, A549 (lung cancer cells) seems to be almost unaffected by hyperthermia when the experimental temperature was raised to 43 °C or more. This phenomenon with caspase 3 induced by the treated cancer cells has also been confirmed in western blot analysis as shown in Fig. 3 compared to the control (Fig. 3).

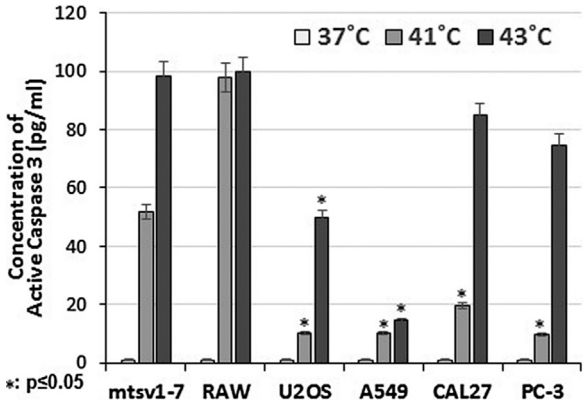

Fig. 2. Analysis of hyperthermia-mediated caspase 3. 100 μl supernatant from the 1 h treatment (37 °C, 41 °C or 43 °C) above (Table 1) was collected and analyzed by ELISA to detect caspase 3 concentration. The caspase 3 concentration of each test group (41 °C or 43 °C) was then compared to the effect of the negative control group (37 °C). Data are graphed and their compared significance is indicated.

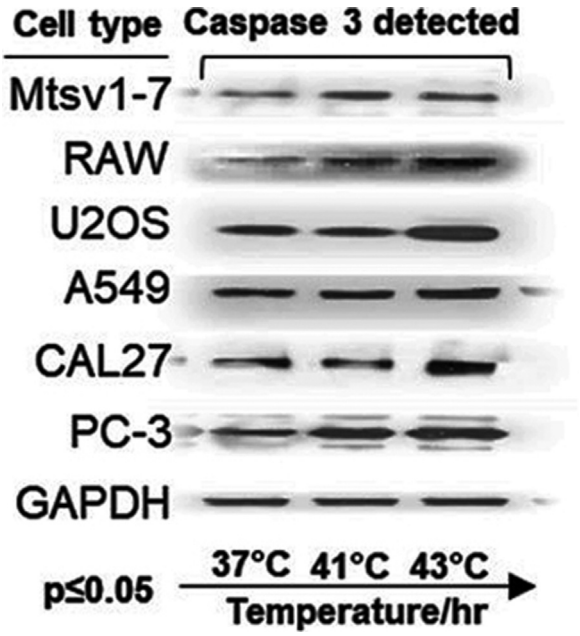

Fig. 3. Western analysis. Cells were treated at 37 °C, 41 °C or 43 °C for 1 h, then the proteins from each test group were purified and subjected to western blot analysis with antibody against caspase 3 or GAPDH as control.

4. Discussion

Treatment of cancer often includes surgical resection, chemotherapy and radiation therapy.[11] Unfortunately, the overall survival rate of treatment remains low and clinical outcomes could still be improved. Thus, new therapeutic strategies need to be developed. Recently, postoperative hyperthermia has been used as an important adjuvant therapy to improve the efficacy of traditional chemotherapy and/or radiotherapy.[12,13] In clinical studies of postoperative hyperthermia, water baths are often used in heating systems, and their temperature is the only indicator evaluated.[14,15] However, the exact temperatures that allow cancer cells to undergo apoptosis without affecting normal cells are unclear. There has been no targeted research and effective statistics. Therefore, one of the purposes of this study was to use a water bath heating system to study the effects of different temperatures on various cancer cells and to explore potential underlying mechanisms.

Recently, research groups have discovered that cancer cells significantly undergo apoptosis by hyperthermia[16] mostly via caspase 3,[8–10,17] with minor involvement of other factors, such as protein 70/90, TRAIL, or ROS/JNK, which are also found to be enhanced by hyperthermia.[18–20] Thus, this paper just focuses on the involvement of caspase 3 in hyperthermia-mediated cells apoptosis.

Our data here indicates that cancer cells in a hyperthermic environment are more resistant to higher temperatures than normal cells. Indeed, we found that human macrophage (THP-1) cells result in 100% death at 41° for 1 h (data not shown). Therefore, a major problem has occurred that cannot be ignored in the hyperthermia treatment of cancer/tumor patients, namely, that hyperthermia not only fails to induce apoptosis in cancer/tumor cells, but may also damage more immune cells that inhabit the bloodstream in the process. This is a significant issue that needs to be brought to attention as the medical community has always believed that a high temperature treatment can selectively kill cancer/tumor cells, which can prevent formation of malignant metastasis and recurrence of cancer/tumor cells in cancer patients.

Interestingly, we found that hyperthermia-treated (≥41°) cancer cells extend a long pseudopod-like extension (Fig. 4a) in comparison to the same cancer cells (Fig. 4b) under normal conditions (37 °C). We believe the pseudopod-like extensions of hyperthermia-treated cancer cells must be related to its resistance to hyperthermia but its function is unknown. We hypothesize that inhibition of cancer cells pseudopod-like extensions may help to damage and kill cancer cells/tumor during hyperthermia treatment. We will figure out this question at a later time.

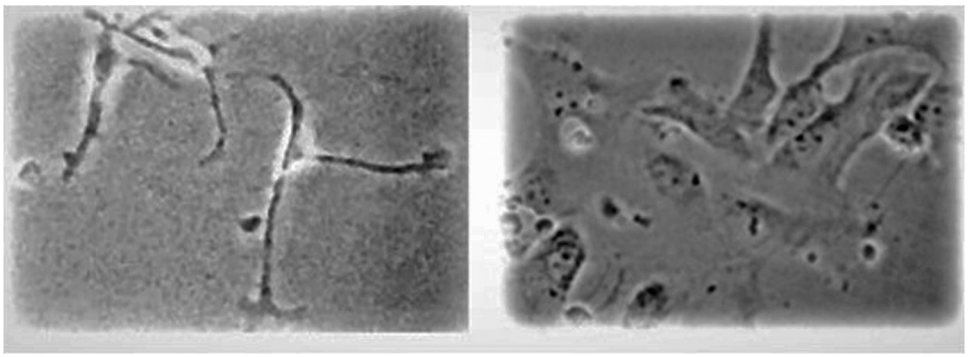

Fig. 4. Effect of hyperthermia on the morphological characteristics in U2OS cancer cells. The pre-cultured U2OS cells with ≥90% cell confluent in the well of 6 well/plate were washed with pBS and treated with 250 μl of 0.25% trypsin for 5 min, then mixed with 2 ml of cell culture medium. Cells were collected, transferred into the sterilized 15 ml tube and incubated in a water bath at 37 °C (A) (control group) or 41 °C (B) (test group) for 1 h. The treated cells with medium were re-plated into one well of a six well/plate and cultured at 37 °C, 5%CO_2 for 24 h. Photomicrograph of cells was taken at ×40 magnification.

Conflicts of interest

None declared.

Financial disclosure

None declared.

References

1. Mahmood J, Shukla HD, Soman S, et al. Immunotherapy, radiotherapy, and hyperthermia: A combined therapeutic approach in pancreatic cancer treatment. *Cancers (Basel)*. 2018;10:12, http://dx.doi.org/10.3390/cancers10120469, pii: E469.
2. Amaya C, Kurisetty V, Stiles J, et al. A genomics approach to identify susceptibilities of breast cancer cells to fever-range hyperthermia. *BMC Cancer*. 2014;14:81.
3. Jose J, Kumar R, Harilal S, et al. Magnetic nanoparticles for hyperthermia in cancer treatment: An emerging tool. *Environ Sci Pollut Res Int*. 2019;(December 28), http://dx.doi.org/10.1007/s11356-019-07231-2.
4. Kristian Storm F, Harrison WH, Elliott RS, Morton DL. Normal tissue and solid tumor effects of hyperthermia in animal models and clinical trials. *Cancer Res*. 1979;39:2245–2251.
5. Yarmolenko Pavel S, Moon EJ, Landon C, et al. Thresholds for thermal damage to normal tissues: An update. *Int J Hyperthermia*. 2011;27:320–343.
6. Moise S, Byrne JM, El Haj AJ, Telling ND. The potential of magnetic hyperthermia for triggering the differentiation of cancer cells. *Nanoscale*. 2018;10:20519–20525.
7. Zhu S, Wang J, Xie B, Luo Z, Lin X, Liao DJ. Culture at a higher temperature mildly inhibits cancer cell growth but enhances chemotherapeutic effects by inhibiting cell-cell collaboration. *PLoS One*. 2015;10:e0137042.
8. Fukuda H, Tomimatsu T, Kanagawa T, et al. Postischemic hyperthermia induced caspase-3 activation in the newborn rat brain after hypoxia-ischemia and exacerbated the brain damage. *Biol Neonate*. 2003;84:164–171.
9. Zhao YY, Wu Q, Wu ZB, et al. Microwave hyperthermia promotes caspase-3-dependent apoptosis and induces G2/M checkpoint arrest via the ATM pathway in non-small cell lung cancer cells. *Int J Oncol*. 2018;53(August (2)):539–550.
10. Mirkes PE, Little SA, Umpierre CC. Co-localization of active caspase-3 and DNA fragmentation (TUNEL) in normal and hyperthermia-induced abnormal mouse development. *Teratology*. 2001;63:134–143.
11. Torre LA, Siegel RL, Jemal A. Lung cancer statistics. *Adv Exp Med Biol*. 2016;893:1–19.
12. Issels RD, Lindner LH, Verweij J, et al. Neo-adjuvant chemotherapy alone or with regional hyperthermia for localised high-risk soft-tissue sarcoma: A randomised phase 3 multicentre study. *Lancet Oncol*. 2010;11:561–570.
13. Datta NR, Rogers S, Ordóñez SG, Puric E, Bodis S. Hyperthermia and radiotherapy in the management of head and neck cancers: A systematic review and meta-analysis. *Int J Hyperthermia*. 2016;32:31–40.
14. Lee H, Park HJ, Park CS, et al. Response of breast cancer cells and cancer stem cells to metformin and hyperthermia alone or combined. *PLoS One*. 2014;9:e87979.
15. Gao F, Ye Y, Zhang Y, Yang J. Water bath hyperthermia reduces stemness of colon cancer cells. *Clin Biochem*. 2013;46:1747–1750.
16. Pawlik A, Nowak JM, Grzanka D, Gackowska L, Michalkiewicz J, Grzanka A. Hyperthermia induces cytoskeletal alterations and mitotic catastrophe in p53-deficient H1299 lung cancer cells. *Acta Histochem*. 2013;115:8–15.
17. Esmaeili Govarchin Ghaleh H, Zarei L, Mansori Motlagh B, Jabbari N. Using CuO nanoparticles and hyperthermia in radiotherapy of MCF-7 cell line: Synergistic effect in cancer therapy. *Artif Cells Nanomed Biotechnol*. 2019;47:1396–1403.
18. Haghniaz R, Umrani RD, Paknikar KM. Hyperthermia mediated by dextran-coated La0.7Sr0.3MnO3 nanoparticles: In vivo studies. *Int J Nanomedicine*. 2016;11:1779–1791.
19. Zhang ZQ, Song SC. Multiple hyperthermia-mediated release of TRAIL/SPION nanocomplex from thermosensitive polymeric hydrogels for combination cancer therapy. *Biomaterials*. 2017;132(July):16–27.
20. Jin H, Zhao Y, Yang J, Zhang X, Ma S. Hyperthermia enhances the sensitivity of pancreatic cancer SW1990 cells to gemcitabine through ROS/JNK signaling. *Oncol Lett*. 2018;16:6742–6748.